New Approaches to Chikungunya Virus Vaccine Development

Alexis Garcia*, Lema Diego and Barroso Judith

Instituto de Inmunología, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela, FOCIS Center of Excellence, Caracas, Venezuela

Received: October 13, 2014; Accepted: January 15, 2015; Revised: February 9, 2015

Abstract: Chikungunya virus (CHIKV) is a mosquito-borne human pathogen that affects millions of individuals each year by causing non-specific flu-like symptoms, with a characteristic rash accompanied by joint pain that may last for a long time after the resolution of the infection. Despite intense research efforts, no approved vaccine or antiviral therapy is yet available. This review is based on articles retrieved by PubMed and clinical trials since 1980 to present. Virus complexity, protective and non-protective immune responses against the virus, and the most important a new patented approaches for Chikungunya vaccine development are discussed.

Keywords: Chikungunya virus, DNA vaccines, immune response, vaccines, virus particles, viral vectors.

INTRODUCTION

Firstly described in the 1950’s, Chikungunya virus (CHIKV) is an arbovirus belonging to the genus Alphavirus (Togaviridae family). It has a single-stranded RNA genome of positive polarity encoding four nonstructural (nsP1-4) and three structural proteins (C, E1, E2). The CHIKV is transmitted by the mosquitoes of the species Aedes albopictus and Aedes aegypti [1].

Once the CHKV is inside the host cell, it replicates at a high rate and induces its cytopathic effect. The molecular mechanism of the debilitating syndrome is not well understood.

The clinical onset is sudden after a silent incubation period lasting from 1 to 12 days, with high fever, headache, back pain, myalgia, and arthralgia, which can be intense, affecting mostly the small joints (ankles, wrists, phalanges) but also the large joints. Skin manifestations in the form of pruriginous maculopapular rash have been recorded in about 40-50% of cases. The hallmark of Chikungunya rarely affecting children is an unpredictable, relapsing, and incapacitating arthralgia which may persist for several months [2].

The pathogenesis of CHIKV infection in humans is still poorly understood, but recent studies have provided insights into the cells and organs involved in viral replication. CHIKV can replicate and induce cytopathic effect in human epithelial and endothelial cells, primary fibroblasts and to a lesser extent, monocyte-derived macrophages [2].

Currently, even though there is no effective vaccine available against CHIKV, several vaccine candidates are being evaluated. This review is focused on the development of new vaccines and patents for the prevention of CHIKV infection.

CHIKV IMMUNE INTERACTION

Innate Immune Response to CHIKV

A wide range of cytokines, chemokines and growth factors are produced during CHIKV infection [3]. Ng and collaborators [4] evaluated an ample range of systemic cytokine production. A significant boost in IL-1β, IL-6 and a decline in Regulated on Activation Normal T Cell Expressed and Secreted (RANTES) secretion were associated with disease severity [4]. Chirathaworn et al. [5], assessing IL-18 and IL-18 binding protein serum levels of patients with CHIKV infection (acute or convalescent period), showed that both cytokines levels were increased in patients as compared to controls, suggesting that Chikungunya virus infection promotes the T helper-1 response by means of IL-18 production [5]. Hoarau et al. [6] reported that the transcription of IFNγ and IL-12 were significantly higher in the peripheral blood mononuclear cells of chronic patients and in the synovial tissue of CHIKV chronic infected patients [6]. Couderc et al. [7] demonstrated that CHIKV infection severity is critically dependent of age and functionality of type-I IFN signaling; neonatal mice as well as adult mice harboring one or two copies of IFN- α/β receptor null allele are more susceptible...
to develop a mild or severe infection compared to wild type mice. Schilte et al. [8] based on their findings in human studies and mouse experimentation concluded that nonhematopoietic cells are important players for IFN production and viral control.

A phenotypic and functional study of natural killer (NK) cells was assessed in patients during acute infection with CHIKV [9]. Petidemange et al. [9] encountered a decreased number of CD3+CD56+ circulating NK cells in CHIKV-infected patients as compared to controls (p < 0.001). Nevertheless, the proportions of these cells increased significantly after CHIKV infection, and consequently, the percentage of activated NK cells was higher in patients (p < 0.0001) as compared to controls. The authors demonstrated that CHIKV infection can selectively shape the natural killer receptor repertoire of healthy individuals [9].

Adaptive Immune Response to CHIKV

Increased production of aforementioned immune mediators indicates the engagement of the adaptive immune response. Wauquier et al. [10], showed a prompt cellular response during human acute CHIKV infection, characterized by up-regulation of CD3+ CD8+ cells, and a down-regulation of CD3+ CD4+ cells in the first two days of symptoms [10].

Humoral immune response has also been evaluated. Human antibodies isolated from the plasma of a CHIKV convalescent patient, have been shown to both prevent and cure CHIKV infection in mice [11]. Kam et al. [12], carried out a longitudinal analysis of the human antibody response using plasma from patients at different times post infection, finding that the E2 glycoprotein is the primary target for the anti-CHIKV antibody response during the complete course of the disease and also that multiple linear regions within the CHIKV proteome are recognized by specific anti-CHIKV antibodies [12]. Lee et al. [13], described CHIKV mutants that escape antibody-dependent neutralization. Additionally, they demonstrated CHIKV direct cell-to-cell transmission [13].

VACCINES

Spread of CHIKV infection could be prevented with effective mosquito control, but this has proven to be difficult. The most effective preventive measure to control the infection is vaccination. Although no licensed Chikungunya prophylactic vaccine is yet available, several vaccine candidates are currently under development.

ATTENUATED VIRUS VACCINES

Live attenuated vaccines contain weakened forms of the virus; historically they have been the most effective intervention in the fight to prevent infectious diseases. In addition to their efficacy, live attenuated vaccines are relatively inexpensive to produce, making them especially useful in resource-limited countries where CHIKV fever is endemic.

In 1984, the Salk Institute-Government Services Division manufactured the first lot of live attenuated vaccine against CHIKV [14]. It was produced from CHIKV strain 15561 previously isolated from a patient in Thailand, which was subjected to several passages until finally obtained CHIKV 181/25 clone. This clone was subsequently evaluated by Levitt et al. [15], in suckling mice and rhesus monkeys observing the appearance of measurable neutralizing antibodies which protected both [15]. In the 1980’s, five clinical trials Phase I were conducted with this vaccine regarding evaluation of safety and immunogenicity, being reported as highly immunogenic, with 98% of the subjects (129/131) developing neutralizing antibodies to CHIKV after a single dose. The main safety issues were transient arthralgia and arthritis [15]. Thereafter, Edelman et al. [16], conducted a Phase II study in 73 healthy adult volunteers; safety profile was similar to the previous Phase I studies, 98% of vaccinated subjects seroconverted by Day 28. Neutralizing antibodies (NAb’s) were still detected in 85% of vaccinated volunteers after 1 year [16]. In spite of its robust immunogenicity, CHIKV 181/25 clone vaccine production was halted because it was not a priority at that time. Gardner et al. [17], identified a group of E2 mutations which confer reduced virulence in a murine model of musculoskeletal disease, CHIKV with the E2-79K mutation having the highest degree of attenuation, allowing its use for future live attenuated candidate vaccines [17].

An internal ribosome entry site (IRES), is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA sequence as part of the greater process of protein synthesis; this technology has been used to attenuate virus belonging to Flaviviridae family [18]. Plante et al. [19], designed an IRES-based vaccine for CHIKV, it was tested in several murine models; they demonstrated that this vaccine was immunogenic, effective and unable to infect mosquitoes. Roy and coworkers [20] developed two live attenuated candidate vaccines using the aforementioned technology. Cynomolgus macaques were vaccinated with a single dose of either vaccine. Approximately 52 days after vaccination, the macaques were challenged with wild-type CHIKV strain, La Reunion. Independent of vaccine strain or route of administration, all vaccinated macaques developed NAb’s to CHIKV; both candidate vaccines elicited remarkably protection from acute viremia [20].

Hallengärd et al. [21] constructed two novel attenuated CHIKV clones by deleting large part of the gene encoding nsP3 or the entire gene encoding 6K. Both genes are crucial in the processes of replication, formation and budding of the CHIKV. After a single dose, they induced high titer of NAb’s as well robust T cell response that protected mice from
viremia and joint swelling. This vaccine could be tested in humans in the near future.

**VIRUS-LIKE PARTICLES VACCINES**

Virus-like particles (VLPs), which resemble infectious virus particles in structure and morphology, have been proposed to provide a new generation of vaccine candidates against various viral infections [22]. As effective immunogens, characterized by high immunogenicity and safety, VLPs have been employed in the development of the two licensed human papilloma virus vaccines [22].

A VLP-based vaccine from strain 37997, expressing the CHIKV envelope proteins, produced high-titered neutralizing antibodies in monkeys after three doses, and protected them against viremia after challenge [23]. Metz et al. [24] evaluated insect cell-derived CHIKV-subunit and VLP vaccine candidates; mice AG129 were vaccinated twice with subunits or VLPs that were formulated in Matrix M (Isconova) adjuvant, latterly they were challenged with a lethal dose of the CHIKV-S27 isolate. The results of the study led to the conclusion that CHIKV VLPs developed high neutralizing antibody titers and provided complete protection against lethal CHIKV challenge [24].

In August 2014, the results of a study Phase I conducted by Chang and coworkers were published [25]. The vaccine consists of Chikungunya VLPs composed of the E1, E2, and capsid proteins from the Chikungunya virus strain 37997. Twenty five subjects participated in a dose escalation trial, 10µg (n = 5), 20µg (n = 10) or 40µg (n = 10), each subject received three doses. The vaccine was generally well tolerated and exhibited good safety profile [25]. According to immunogenicity assays, all participants developed NAb after the second dose, which remained detectable up to 6 months after the last dose [25].

**VIRAL VECTORS VACCINES**

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. Viral-vector vaccines remain the best means to induce cellular immunity and are now showing promise for the induction of strong humoral responses [26].

In 2011, Wang et al. [27] assessed in mice the non-replicating complex adenovirus vaccine vectors expressing the envelope glycoproteins E1, E2 and capsid of CHIKV. Vaccine was able not only to elicit high titres of NAb but also protected mice against viremia and arthritic disease [27]. Brandler and collaborators [28], developed a recombinant Measles Virus-CHIKV virus expressing CHIKV VLPs patented recently [29]. Their strategy was based on the use of a replicating vector that results in the production and diffusion through the organism of CHIKV VLPs at the time of vaccination. The vaccine was evaluated in genetically modified mice, inducing high levels of NAb, which were able to protect them after lethal challenge with CHIKV [28].

Recombinant vesicular stomatitis virus (rVSV) is currently under evaluation [30]. Numerous preclinical studies with rVSV vectors expressing antigens from a variety of human pathogens have demonstrated the versatility, flexibility, and potential efficacy of the rVSV vaccine platform [30]. Chattopadhyay et al. [31], in 2013, generated an experimental vaccine which contained rVSV expressing CHIKV envelope polyprotein (E3-E2-6K-E1). Chimeric viruses were inoculated in mice; 34 days after immunization they were challenged with wild CHIKV. After a single dose, mice elicited a strong immune response characterized by high levels of NAb and protection from challenge [31].

Recombinant Modified Vaccinia virus Ankara (MVAs) expressing E3E2, 6KE1, or the entire CHIKV envelope polyprotein cassette E3E26KE1 was evaluated in mice susceptible to CHIKV infection [32]. Each animal received two doses; subsequently they were challenged with CHV-S27. All mice immunized with MVAs expressing E2 or E3E26KE1 produced NAb which provided 100% protection against lethal disease [32]. Garcia-Arriaza and collaborators [33] evaluated another MVA-CHIKV chimera expressing C, E3, E2, 6K and E1 genes in female C57BL/6 mice. This chimera induced a robust adaptive response, generating CHIKV-specific CD8+ T-cells and high titers of NAb [33]. Seven weeks after the last dose mice were challenged; vaccinated mice did not develop viremia or inflammation signs [33].

**DNA VACCINES**

DNA vaccination involves cloning the gene(s) of interest into a plasmid backbone and delivering the DNA through different routes of immunization [34]. The DNA is taken up by cells, the protein of interest is expressed, and antigen-presenting cells take the antigen to the draining lymph nodes. DNA vaccination results in antigen expressed by both MHC class I and class II, leading to activation of CD8+ and CD4+ T cells, as well as antibody responses [34].

In 2008, Muthumani et al. [35], presented data of a novel consensus-based approach to vaccine design for CHIKV, employing a DNA vaccine strategy. This vaccine was composed of consensus sequence Capsid E1 and E2, enhancing expression with an IgE leader sequence and a strong Kozak sequence [35]. This vaccine proved to be immunogenic in vivo after intramuscular delivery via electroporation in mice, after two doses stimulating both cellular and humoral response, thus proving DNA vaccines to be promising agents in CHIKV protection [35]. Mallilankaraman et al. [36], developed a CHIKV DNA vaccine which included all three envelope protein genes (E1, E2 and E3) but no Capsid gene, as well as an IgE leader sequence, a Kozak sequence and furin cleavage sites between envelope sequences to facilitate...
protein processing. Mice immunized with this vaccine showed robust cellular and humoral immune response (including NAbs) and protection against a CHIKV challenge [36]. This vaccine also proved to be immunogenic in rhesus macaques, eliciting cellular and humoral responses; they were not challenged with the virus to assess protection, but their response to the vaccine was similar to that of convalescent humans [36]. Later, in 2013, Bao and collaborators [37] added the nonstructural protein-2 (nsP2) as adjuvant to envelope (E2) DNA vaccine. This formulation significantly increases both cellular and humoral immune responses in particular specific NAbs and protection of mice against viral challenge [37].

Recently, a novel immunization DNA vaccination strategy has yielded a CHIKV vaccine which has overcome some of the previous DNA vaccines limitations [38]. The new strategy is based on plasmid DNA that encodes the full-length infectious genome of live attenuated CHIKV clone 181/25 [38]. All BALB/c mice immunized with this vaccine seroconverted and showed protection against infection, however further experiments are required.

NEW PATENTS

Current efforts have been directed to develop a safe and effective vaccine. Inactivated virus was the older approach [39] which recently has had new modifications [39] based on genetic diversity and serotype analysis in India [40]. However, inconveniences with virus purifications and mutations in culture lead new approaches.

Pushko et al. [41] proposed the use of i-DNA vectors for pathogenic RNA viruses inducing a live attenuated virus capable of inducing a strong immune response in the host against the wild type virus. Even though this last technique has not been documented for CHIKV, the results with Venezuelan equine encephalitis [42] and yellow fever look promising [43].

Taking into account the importance of having high quantities of reliable effective vaccines, Brown and Hernández [44] proposed an interesting element using insect cells to generate attenuated virus with modified CHIKV containing deletions in the E2 protein making them less prone to be infective, but at the same time effective in immune response against the virus.

The use of VLPs has also been assayed using different approaches. Ella and Kandaswamy [45] designed a particle containing a variety of viral antigens delivered through VLPs which should be suitable for protecting against genotype variants, a strategy which differs from the previous patents although with similar scopes [39].

As described previously, Tangy and coworkers [29] generated a vaccine using recombinant Measles virus expressing CHIKV envelope and capsid proteins of CHIKV [28]. The particles are able to replicate in the host an interesting strategy for long term immune response with single inoculations as proposed by Pushko et al. [42]. In similar fashion, Pugachev [46] has described a method which would enhance the use of replication defective flavivirus using the RepliVax platform which seem to have promising results in several flavivirus based vaccines [47]. However, the results of the chimeric CHIKV vaccine developed by Frolov et al. [48] using alphavirus DNA combined with CHIKV have not been documented. Thus, further analyses are required for assessing vaccine safety and effectiveness.

The only effort using immunogenic peptides was developed by Tong and coworkers [49] based on the previous reports [12, 50] of early neutralizing IgG response against the virus and the different epitopes of the E2 protein. Even though the effort was interesting, the previous designs with modified E2 proteins using virus like particles or other structures seem to be more effective.

In general, the different patents have provided a new element to analyze immune response against the virus and provide new ways of vaccination which could benefit large populations in underdeveloped countries suffering this disease.

CURRENT & FUTURE DEVELOPMENTS

Chikungunya Virus disease (CHIKVD) is currently an expanding global public health problem that needs a global strategy besides vector control which has achieved only limited success in reducing the transmission of CHIKV and facing the fact that there are currently no licensed antivirals to treat CHIKVD. Even CHIKV is not considered life-threatening; it has high morbidity impact in patients in an acute and long term setting. The most effective way to control CHIKVD in the future will include the use of a safe and effective vaccine. Three decades ago began considerable progress in the development of a CHIKV vaccine with many promising efforts. Most major vaccine approaches have been applied recently to CHIKV, but further efforts are needed in order to find the most suitable vaccine. Current actions have been directed to develop a safe and effective vaccine. From new modifications for the inactivated virus approach that look promising, to different scopes such as the new strategies of recombinant vaccines seem to be highly effective in generating an effective immune response against the virus. Table 1 illustrates relevant vaccine strategies already developed. Most probably, they could render effective results in neglected tropical diseases in which vaccine efforts have been limited due to the lack of financial support. Changes in the mosquito’s ecosystem plus uncontrolled migration may influence the spreading and increase the possible mutations of the virus to make it resistant to pharmacologic therapies. Thus, vaccines are excellent tools not only in the decrease the propagation of the disease, but also enhancing effective
immune response protecting general populations and reducing morbidity and mortality as encountered for diseases like polio and measles. A bright future may be closer than expected.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The study was financed by FONACIT-Venezuela G-2005000389.

REFERENCES


